

IN THE CLAIMS:

1-171. (cancelled).

172. (previously presented) A method for making a transcription product having a sequence corresponding to a target sequence in a target nucleic acid in a sample, the method comprising the steps of:

- (a) obtaining an RNA polymerase that can transcribe RNA using a single-stranded promoter;
- (b) obtaining single-stranded DNA comprising the target sequence that is present in or complementary to a sequence in the target nucleic acid in the sample;
- (c) operably joining to the single-stranded DNA a single-stranded polynucleotide comprising a promoter that binds the RNA polymerase, thereby obtaining a single-stranded transcription substrate;
- (d) obtaining nucleoside triphosphates (NTPs) that are substrates for the RNA polymerase and that are complementary to canonical nucleic acid bases;
- (e) admixing the RNA polymerase, the single-stranded transcription substrate and the NTPs; and
- (f) incubating the RNA polymerase, the single-stranded transcription substrate and the NTPs to synthesize the transcription product.

173. (previously presented) The method of claim 172, the method additionally comprising the steps of:

- (g) obtaining a reverse transcriptase;
- (h) reverse transcribing the transcription product from step (f) to obtain a first-strand cDNA complementary to the transcription product;
- (i) operably joining to the first-strand cDNA a single-stranded polynucleotide comprising a promoter that binds the RNA polymerase, thereby obtaining a second single-stranded transcription substrate;
- (j) admixing the RNA polymerase, the second single-stranded transcription substrate and the NTPs; and
- (k) incubating the RNA polymerase, the second single-stranded transcription substrate and

the NTPs to synthesize a second transcription product.

174. (previously presented) The method of claim 172, wherein the single-stranded DNA comprising the target sequence is obtained using a target nucleic acid comprising: (a) DNA; (b) at least one mRNA; or (c) substantially all mRNA in a sample.

175. (previously presented) The method of claim 172, wherein the single-stranded transcription substrate of step (c) is obtained by primer extension of the single-stranded DNA of step (b) using a promoter splice template oligo annealed to the 3'-end of the single-stranded DNA as a template, said splice template oligo comprising: (a) a 5'-end portion that is complementary to a desired sequence to be added to the 3'-end of the first-strand cDNA; and (b) a 3'-end portion that is complementary to the 3'-end of the first-strand cDNA, wherein the 3'-terminus is blocked so it cannot be primer extended using a DNA polymerase.

176. (previously presented) The method of claim 175, wherein the 5'-end portion of said splice template oligo is complementary to part of or all of a sense or an anti-sense promoter sequence for an RNA polymerase that can bind a single-stranded promoter.

177. (previously presented) The method of claim 175, wherein the single-stranded DNA is obtained by reverse transcription of a transcription product.

178. (previously presented) The method of claim 175, wherein steps (b) and (c) comprise the sub-steps of:

- (a1) obtaining a primer for synthesis of a first-strand cDNA, the primer comprising a sequence complementary to the sequence at the 3'-end of the target sequence to be transcribed;
- (b1) annealing the primer to the target nucleic acid;
- (c1) primer-extending the primer annealed to the target nucleic acid with a DNA polymerase to obtain a linear first-strand cDNA comprising a sequence complementary to the target sequence;
- (d1) obtaining a promoter splice template oligo comprising:
 - (i) a 3'-end that is sufficiently homologous to the 3'-end of the linear first-strand

cDNA, including the tail if present, to hybridize therewith, and that is blocked so that it cannot itself be primer-extended by a DNA polymerase; and

(ii) a 5'-portion that exhibits a sequence that is complementary to a transcription promoter for an RNA polymerase that can synthesize RNA using a single-stranded transcription substrate;

(e1) annealing the promoter splice template oligo to the linear first-strand cDNA including the tail if present;

(f1) primer-extending the linear first-strand cDNA including the tail, if present, with a DNA polymerase to obtain a promoter-containing linear first-strand cDNA that has a 3'-portion that is complementary to the portion of the promoter splice template oligo that is not hybridizable to the first-strand cDNA including the tail, if present; and

(g1) removing or dissociating the promoter splice template oligo from the promoter-containing linear first-strand cDNA to obtain the single-stranded transcription substrate.

179. (previously presented) The method of claim 178, additionally comprising the steps of:

(a2) obtaining a blocking oligo, the blocking oligo comprising a sequence that anneals to the target nucleic acid so as to delimit the 3'-end of a primer extension product of the primer using the target nucleic acid as a template, wherein the blocking oligo is not displaced by the primer extension product, and wherein the blocking oligo is not itself capable of being primer-extended by a DNA polymerase; and

(b2) annealing the blocking oligo, together with the primer, to the target nucleic acid in step (b1) prior to primer-extending the primer in step (c1):

180. (previously presented) The method of claim 178, wherein the target nucleic acid that is annealed to the linear first-strand cDNA is removed prior to annealing the promoter splice template oligo to the linear first-strand cDNA.

181. (previously presented) The method of claim 178, wherein a tail is added to the 3'-end of the linear first-strand cDNA prior to annealing the promoter splice template oligo to the linear first-strand cDNA.

182. (previously presented) The method of claim 172, wherein steps (b) and (c) comprise the sub-steps of:

- (a) obtaining a target mRNA;
- (b) obtaining a primer for synthesis of a linear first-strand cDNA that is complementary to the mRNA, the primer selected from the group consisting of:
 - (i) an oligo(dT) primer;
 - (ii) an oligo(dT) anchor primer;
 - (iii) a primer that is complementary to a specific sequence at the 3'-end of the mRNA;and
- (iv) a primer in a mixture of primers, the primer comprising a sequence of nucleotides, each of which nucleotides comprises a random nucleotide base that is complementary to any of the four canonical nucleotide bases;
- (c) annealing the primer to the target mRNA;
- (d) primer-extending the primer annealed to the target mRNA with a reverse transcriptase to obtain a linear first-strand cDNA that is complementary to and annealed to the target mRNA;
- (e) obtaining a promoter splice template oligo comprising:
 - (i) a 3'-end that is sufficiently homologous to the 3'-end of the linear first-strand cDNA including the tail, if present, to hybridize therewith, and that is blocked so that it cannot itself be primer-extended by a DNA polymerase; and
 - (ii) a 5'-portion that exhibits a sequence that is complementary to a transcription promoter for an RNA polymerase that can synthesize RNA using a single-stranded transcription substrate;
- (f) annealing the promoter splice template oligo to the linear first-strand cDNA including the tail, if present;
- (g) primer-extending the linear first-strand cDNA including the tail, if present, with a DNA polymerase to obtain a promoter-containing linear first-strand cDNA that has a 3'-portion that is complementary to the portion of the promoter splice template oligo that is not hybridizable to the linear first-strand cDNA including the tail, if present; and
- (h) removing or dissociating the promoter splice template oligo from the promoter-containing linear first-strand cDNA to obtain the single-stranded transcription substrate.

183. (previously presented) The method of claim 182, wherein the target mRNA that is annealed to the linear first-strand cDNA is removed prior to annealing the promoter splice template oligo to the linear first-strand cDNA.

184. (previously presented) The method of claim 182, wherein a tail is added to the 3'-end of the linear first-strand cDNA prior to annealing the promoter splice template oligo to the linear first-strand cDNA.

185. (previously presented) The method of claim 172, wherein the transcription product is used to obtain an additional single-stranded transcription substrate and an additional transcription product comprising a sequence corresponding to the target sequence, the method comprising the sub-steps of:

- (a1) obtaining a primer for synthesis of a first-strand cDNA, the primer exhibiting a sequence that is complementary to the sequence at the 3'-end of the transcription product;
- (b1) annealing the primer to the transcription product;
- (c1) primer-extending the primer annealed to the transcription product with a DNA polymerase to obtain a linear first-strand cDNA that is complementary to and annealed to the transcription product;
- (d1) obtaining a promoter splice template oligo comprising:
 - (i) a 3'-end that is sufficiently homologous to the 3'-end of the linear first-strand cDNA including the tail, if present, to hybridize therewith, and that is blocked so that it cannot itself be primer-extended by a DNA polymerase; and
 - (ii) a 5'-portion that exhibits a sequence that is complementary to a transcription promoter for an RNA polymerase that can synthesize RNA using a single-stranded transcription substrate;
- (e1) annealing the promoter splice template oligo to the linear first-strand cDNA;
- (f1) primer-extending the linear first-strand cDNA with a DNA polymerase to obtain a promoter-containing linear first-strand cDNA that has a 3'-portion that is complementary to the portion of the promoter splice template oligo that is not hybridizable to the linear first-strand cDNA;
- (g1) removing or dissociating the promoter splice template oligo from the promoter-containing

linear first-strand cDNA to obtain the additional single-stranded transcription substrate; and
(h1) contacting the additional single-stranded transcription substrate from step (h) with an RNA polymerase that transcribes the additional single-stranded transcription substrate using the promoter to obtain the additional transcription product.

186. (previously presented) The method of claim 185, wherein the transcription product that is annealed to the linear first-strand cDNA is removed prior to annealing the promoter splice template oligo to the linear first-strand cDNA.

187. (previously presented) The method of claim 172, wherein steps (b) and (c) comprise the sub-steps of:

(a1) annealing to a single-stranded target nucleic acid a primer complementary to the 3'-end of the single-stranded target sequence;

(b1) extending the primer by reverse transcription or primer extension with a DNA polymerase so as to obtain a first-strand cDNA that is complementary to the target sequence;

(c1) annealing a promoter splice template oligo to the 3'-end of the first-strand cDNA, wherein the promoter splice template oligo comprises:

(i) a 3'-portion that is hybridizable to the 3'-end of the first-strand cDNA including the tail, if present, and that is blocked so that it cannot itself be primer-extended by a DNA polymerase; and

(ii) a 5'-portion exhibiting a sequence that is complementary to a transcription promoter for an RNA polymerase that can synthesize RNA using a single-stranded transcription substrate;

(d1) extending the first-strand cDNA including the tail, if present, by reverse transcription or primer extension with a DNA polymerase so as to obtain an anti-sense-promoter-containing first-strand cDNA that has a 3'-portion that is complementary to the portion of the promoter splice template oligo that is not hybridizable to the target nucleic acid sequence including the tail, if present;

(e1) removing or dissociating the target nucleic acid from the anti-sense-promoter-containing first-strand cDNA;

(f1) circularizing the anti-sense promoter-containing first-strand cDNA with a ligase;

- (g1) annealing to the circular anti-sense-promoter-containing first-strand cDNA a strand-displacement primer, wherein the strand-displacement primer is complementary to a portion of the anti-sense-promoter-containing first-strand cDNA;
- (h1) incubating the circular anti-sense-promoter-containing first-strand cDNA to which the strand-displacement primer is annealed with a strand-displacing DNA polymerase to obtain a linear promoter-containing second-strand cDNA, wherein the linear promoter-containing second-strand cDNA comprises a single-stranded transcription substrate; and
- (i1) obtaining the single-stranded transcription substrate.

188. (previously presented) The method of claim 187, wherein a blocking oligo that delimits the 5'-end of the target sequence is also annealed to the target nucleic acid together with the primer in step (a1).

189. (previously presented) The method of claim 187, wherein a tail is added to the 3'-end of the linear first-strand cDNA obtained in step (b1) prior to annealing the promoter splice template oligo to the linear first-strand cDNA.

190. (previously presented) The method of claim 172, wherein steps (b) and (c) comprise the sub-steps of:

- (a1) primer extending a sense promoter primer with a DNA polymerase using the target nucleic acid in the sample as a template to obtain the single-stranded DNA comprising the target sequence, which single-stranded DNA comprises linear promoter-containing first-strand cDNA; and
- (b1) circularizing the linear promoter-containing first-strand cDNA with a ligase, thereby operably joining the single-stranded DNA comprising the target sequence to the promoter to obtain a circular single-stranded transcription substrate.

191. (previously presented) The method of claim 190, the method additionally comprising the step of cleaving the circular single-stranded transcription substrate at a site that is 3'-of the promoter sequence and 5'-of the target sequence to obtain a linear single-stranded transcription substrate.

192. (previously presented) The method of claim 190, wherein the target nucleic acid in the sample comprises RNA such as mRNA, or a transcription product, and the DNA polymerase used for primer extension is an enzyme with reverse transcriptase activity.

193. (previously presented) The method of claim 172, wherein the single-stranded polynucleotide of step (c) is a promoter ligation oligo and the joining is by ligation using a ligation splint.

194. (previously presented) The method of claim 172, wherein the target sequence has a tail sequence comprising at least two or between two to ten nucleotides.

195. (previously presented) The method of claim 172, wherein at least one of the NTPs is a 2'-amino-deoxynucleoside triphosphate such as 2'-amino-dCTP; a 2'-fluoro-deoxynucleoside triphosphate such as a 2'-fluoro-deoxynucleoside triphosphate selected from 2'-fluoro-dCTP and 2'-fluoro-dUTP; or a 2'-azido-deoxynucleoside triphosphate such as 2'-azido-dCTP.

196. (previously presented) The method of claim 172, wherein the target sequence comprises a 3'-portion that encodes a first portion, a 5'-portion that encodes a second portion that is complementary to the first portion, and a middle portion that joins the 3'-portion and the 5'-portion, wherein the middle portion exhibits a sequence that is not complementary to either the 3'-portion or the 5'-portion and wherein the transcription product comprises a hairpin RNA.

197. (previously presented) The method of claim 196, wherein the hairpin RNA has RNA interference activity in a cell that synthesizes a target mRNA comprising the target sequence.

198. (previously presented) A method for obtaining a substrate for transcription and obtaining a transcription product corresponding to a target sequence using a T7-type RNAP that binds a double-stranded promoter, the method comprising the steps of:

- (a) obtaining a single-stranded target nucleic acid comprising a target sequence;
- (b) obtaining a primer that anneals to the 3'-end of the target sequence;

- (c) annealing the primer to the 3'-end of the target sequence;
- (d) synthesizing a first-strand cDNA by primer extension of the primer annealed to the 3'-end of the target sequence using a DNA polymerase or reverse transcriptase;
- (e) obtaining a splice template oligo exhibiting the anti-sense sequence of a double-stranded promoter, wherein the 3'-end portion of the splice template oligo is capable to anneal to the 3'-end of the first-strand cDNA, including the tail sequence if present, and wherein the 3'-terminal nucleotide of the splice template oligo is a terminator nucleotide such as, but not limited to a dideoxynucleotide;
- (f) annealing the splice template oligo to the first-strand cDNA;
- (g) primer extending the 3'-end of the first-strand cDNA using the annealed splice template oligo as a template using a DNA polymerase or reverse transcriptase;
- (h) obtaining a single-stranded DNA pro-transcription substrate comprising the primer-extended first-strand cDNA exhibiting at its 3'-end a sense promoter sequence;
- (i) annealing to the single-stranded DNA pro-transcription substrate an anti-sense promoter oligo exhibiting an anti-sense promoter sequence complementary to the sense promoter sequence of the pro-transcription substrate;
- (j) obtaining a transcription substrate complex comprising the complex between the single-stranded DNA pro-transcription substrate and the anti-sense promoter oligo; and
- (k) contacting the transcription substrate complex with a cognate T7-type RNAP that binds to the double-stranded promoter in the transcription substrate complex to obtain the transcription product corresponding to the target sequence.

199. (previously presented) The method of claim 198, wherein a tail is added to the 3'-end of the first-strand cDNA obtained in step (d) prior to annealing the splice template oligo to the first-strand cDNA.

200. (previously presented) The method of claim 198, wherein steps (i) and (j) comprise the sub-steps of circularizing the single-stranded DNA pro-transcription substrate using a ligase and annealing the anti-sense promoter oligo, thereby obtaining a circular transcription substrate complex, and step (k) comprises rolling circle transcription of the circular transcription substrate complex using the cognate T7-type RNAP.

201. (previously presented) A method for cloning a target sequence in a target nucleic acid, the method comprising the steps of:

- (a) obtaining a single-stranded DNA comprising the target sequence or a sequence complementary to the target sequence;
- (b) making a circular single-stranded DNA molecule by DNA polymerase-catalyzed primer extension of a primer using the target nucleic acid as a template, followed by circularization of the primer extension product, wherein the primer comprises a single-stranded origin of replication and a marker gene;
- (c) transforming the circular single-stranded DNA molecule into a host cell, in which the marker gene is expressible, wherein the host cell is capable of replicating the circular single-stranded DNA molecule; and
- (d) obtaining a cell clone harboring the circular single-stranded DNA molecule comprising the target sequence.

202. (previously presented) The method of claim 201, wherein the primer of step (b) comprises a promoter primer and, and wherein steps (b) through (d) comprise the sub-steps of:

- (a) obtaining a promoter primer that is single-stranded and comprises: (i) a 3'-end portion exhibiting a sequence complementary to the sequence of the 3'-end portion of the target sequence; and (ii) a 5'-end portion exhibiting a sequence at or near the 5'-end for a sense transcription promoter for an RNA polymerase that can make a transcription product using a single-stranded transcription substrate and additional regions 3'-of the promoter, the additional regions comprising a single-stranded origin of replication that can be replicated in a host cell and at least one gene for a selectable or screenable marker that is expressible in the host cell;
- (b) annealing the promoter primer to the target nucleic acid;
- (c) obtaining a DNA polymerase;
- (d) primer-extending the promoter primer annealed to the target nucleic acid with the DNA polymerase;
- (e) obtaining a linear first-strand cDNA exhibiting a sequence that is complementary to the target sequence;
- (f) circularizing the linear first-strand cDNA by covalently joining the 5'-end of the linear

first-strand cDNA to the 3'-end of the linear first-strand cDNA to obtain circular first-strand cDNA, wherein the circular first-strand cDNA comprises a circular single-stranded transcription substrate;

- (g) obtaining the circular single-stranded transcription substrate;
- (h) obtaining host cells that can replicate a circular single-stranded DNA comprising the single-stranded origin of replication and in which the selectable or screenable marker is expressible;
- (i) incubating the host cells with DNA comprising a circular single-stranded transcription substrate under conditions suitable to obtain transformation;
- (j) plating the host cells with the DNA comprising the circular single-stranded transcription substrate on medium that permits selection or screening for cells that contain and express the gene for the selectable or screenable marker; and
- (k) obtaining transformed host cells comprising the target sequence.

203. (previously presented) The method of claim 202, wherein said origin of replication is an M13 origin of replication.

204. (previously presented) The method of claim 202, wherein (a) the 5'-end of the promoter primer additionally comprises a phosphate group or a topoisomerase moiety; or (b) a phosphate group or a topoisomerase moiety is added to the 5'-end of the linear first-strand cDNA obtained in step (e).

205. (previously presented) The method of claim 202, wherein the target nucleic acid that is annealed to the linear first-strand cDNA is removed prior to circularizing the linear first-strand cDNA in step (f).